NEW SAMPLER FOR THE COLLECTION, SIZING, AND ENUMERATION OF VIABLE AIRBORNE PARTICLES[1, 2]

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Received for publication May 19, 1958

Numerous authors investigating respiratory tract penetration by air particulate pollutants have recognized the relationship existing between particle size and lung penetration. Morton (1956) concluded that the particle size of an aerosol of pathogenic organisms determines the degree of infectivity by the respiratory route. Druet *et al.* (1953) stated that infectivity is greatest with single spore particles and falls off sharply with particles larger than 5 microns. Harper and Morton (1953) said that few particles larger than 4 microns reach the lungs. Brown *et al.* (1950) summarized the theoretical and experimental findings on respiratory tract penetration. They stated that nasal efficiency for screening out airborne particles entering the respiratory tract is practically 100 per cent for particles above 5 microns and decreases with particle size to zero for 1 micron particles; that depth of penetration into the respiratory tract increases with decreasing size; that alveolar retention is complete for particles larger than 1 micron which escape being trapped in the upper respiratory tract; and that from 1 to ¼ micron, alveolar retention decreases. Wells (1955) pointed out that it is not the size of the particles but rather the aerodynamic dimension which determines lung penetrability. From these and many other references it is obvious that any instrument used to assess the health hazard or infection potential of particulate aerosols should determine the number and the size[3] of the airborne particles or, preferably, classify them aerodynamically since penetration and deposition in the respiratory tract is a matter of aerodynamics. A number of instruments (May, 1945; Wilcox, 1953; Armour Research Foundation, 1953; Southern Research Institute, 1955; Sawyer and Walton, 1950), some extremely sensitive and elaborate, have been devised to determine the number and size of airborne particles but none of these devices secures this information for viable bacterial aerosols because they do not differentiate between viable and nonviable particles. It is the objective of this report to describe an instrument with which viable airborne particles are sized and counted, to set forth proper operational procedures, and to present some of the results of experimental studies conducted to date. Although several names have been applied to this device, it is now most generally known as the Andersen (1956) sampler, and was so designated by the Commanding Officer, Biological Warfare Assessment Laboratories, Dugway Proving Ground.

MATERIALS AND METHODS

Description of sampler. A photograph of the latest model of the Andersen sampler is shown in figure 1, and a schematic diagram of the device is presented in figure 2. It can be seen that the instrument consists of a series of six stages through which the sample of air or aerosol is consecutively drawn. The device is pressure sealed with gaskets and three adjustable spring fasteners. Each stage contains a plate perforated with 400 holes (340 in previous models) and immediately below a petri dish of agar culture medium. Air is drawn through the device at the rate of 1 cubic foot per minute (cfm) and a jet of air from each of these holes plays on the sur-

[1] This paper combines an extract of Dugway Proving Ground Research Report 108, *Developmental Work on the Anderson Sampler*, November 1956 (unclassified) with descriptive material of, and studies made with the latest model of the sampler, manufactured for, and distributed by the author, 1074 Ash Ave., Provo, Utah.

[2] Patent has been applied for under the designation Bacterial Aerosol Analyzer, Chemical Corps Patent Board, Item No. 904; Serial No. 569,661.

[3] Size, when used in this paper to refer to the dimensions of particles, is intended to mean aerodynamic dimension. This term takes into account all the properties of the particle that affect its movement in air. Only for smooth, spherical particles can the diameter be measured and the data be useful.

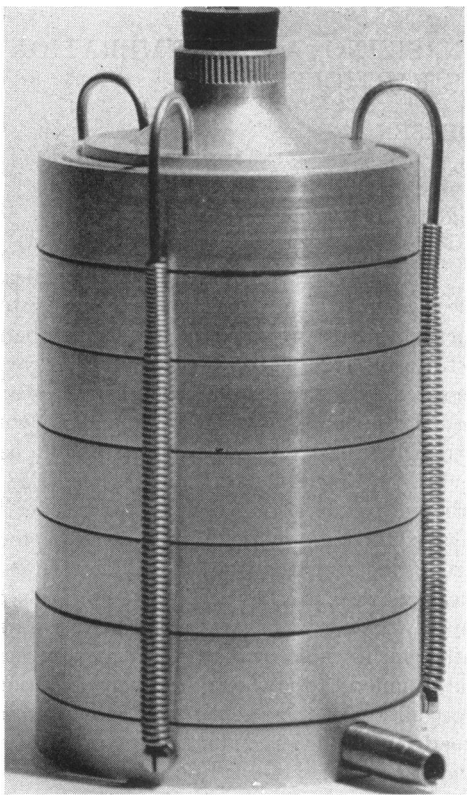

Figure 1. The Andersen sampler

face of the medium. The size of the holes is constant for each stage, but is smaller in each succeeding stage; consequently, the jet velocity is uniform in each stage but increases in each succeeding stage. When the velocity imparted to a particle is sufficiently great, its inertia will overcome the aerodynamic drag and the particle will leave the turning stream of air and be impinged on the surface of the medium; otherwise the particle remains in the stream of air and proceeds to the next stage. The sampler is so designed that when operated at 1 cfm any airborne particle, a fraction of 1 micron or larger, will be collected on one stage or another, depending on its aerodynamic dimensions. If the particle size spectrum of the aerosol sample is sufficiently broad, particles will be collected on all stages of the sampler. Each succeeding stage will remove a top fraction (largest particles) of the remaining particles; the last stage completes the collection of bacterial particles.

The sampler, by the proper selection of hole sizes, may consist of any practical number of stages. The first prototype had four stages, each with 340 holes, and a Millipore filter. The Millipore filter was soon found impractical for many organisms and was replaced with a regular stage containing 340 holes, 0.010 in in diameter. Some sampler stage arrangements have contained as many as nine stages with holes ranging from 0.006 to 0.052 in in diameter, and the U. S. Naval Research Laboratories made a 4 stage modification. The latest model, with hole sizes and jet velocities listed, is shown in figure 2. This model has 400 holes per stage compared with 340 holes in previous models (Andersen, 1956). It has about twice the capacity, especially for fine aerosols, and is much lighter in weight. The sampler is made entirely of aluminum alloy with stainless steel fittings. All aluminum parts are anodized for corrosion resistance. It is 8 in high, 4¼ in in diameter excluding fasteners and outlets and weighs 3⅓ lb without petri plates.

Operation of the sampler. All models of the sampler have been designed to operate at a flow-rate of 1 cfm. This flow-rate may be achieved with a small vacuum pump or with a vacuum system. If a vacuum of 15 in or more of Hg is available, the flow-rate can be controlled with a critical orifice placed in the line near the outlet of the sampler. A piece of capillary tubing 0.75 in in length with a bore of 0.0764 in will serve the purpose. If not controlled with a critical orifice, the vacuum at the outlet of the sampler may be adjusted with a magnehelic gauge to 0.040 in of Hg. The author has designed an electric vacuum pump to operate the sampler at 1 cfm. One unit has been made and tested and it is expected to be in commercial production shortly. There are several other electric vacuum pumps commercially available which are suitable for operating the sampler. These pumps draw slightly more than 1 cfm through the sampler, but can be adjusted to draw the 1 cfm by reducing the bore in the intake of the pump until a wet test meter in front of the sampler indicates proper flow. Rubber tubing with a bore smaller than 5/16 in I.D. or more than 5 ft in length should not be used without checking the flow-rate or the vacuum at the outlet of the sampler.

The collection and assessment of aerosol samples is very simple. Six petri dishes, each containing 27 ml of the agar medium appropriate for the microorganisms which may be encountered, are placed in the instrument and the sam-

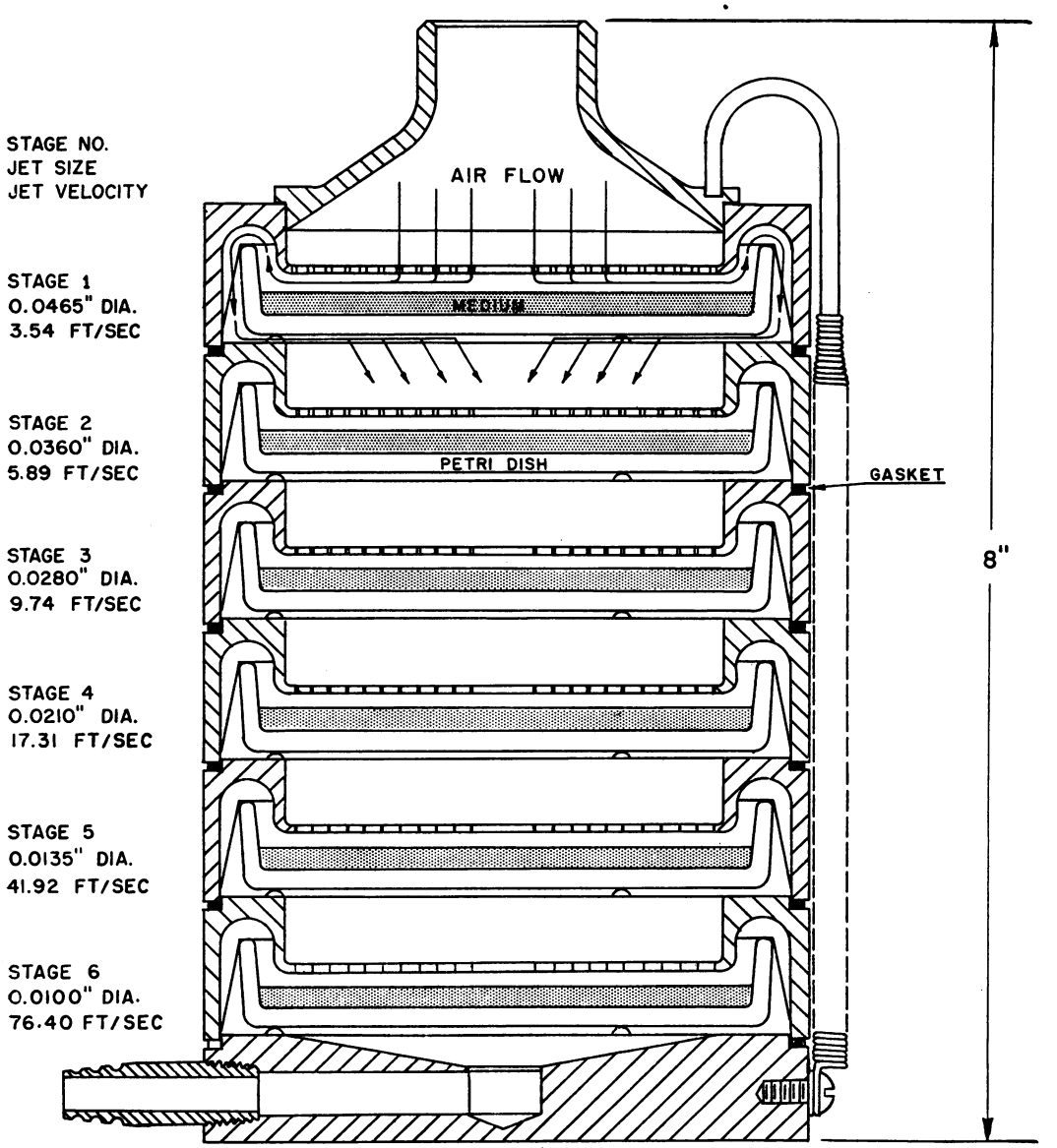

Figure 2. Schematic diagram of a six-stage Andersen sampler

ple of air is drawn; the plates are then removed, inverted in their covers, incubated, and counted by one of the procedures listed below. When samples of aerosols are being drawn from a chamber, air-flow through the instrument should not be interrupted until air washing with clean air from outside the chamber has replaced all of the sample air within the sampler. Air should not be drawn through the sampler unless petri plates are in place because this may lodge dirt in the small holes of the lower stages.

Counting procedures. Colonies on plates 1 and 2 are scattered over the plates and should be counted in the usual manner, except when the plates are heavily loaded, in which case counting may be done through a dissecting type microscope before the colonies merge. A number of fields or segments of the plate are counted, and the total number of colonies for the plate is calculated.

In stages 3–6, the colonies conform to the pattern of jets and are counted by either the

TABLE 1

Positive hole conversion table: Positive hole counts (r) and corresponding corrected particle counts (P)

r	P	r	P	r	P	r	P	r	P	r	P	r	P	r	P	r	P	r	P
1	1	41	43	81	91	121	144	161	206	201	279	241	369	281	485	321	649	361	931
2	2	42	44	82	92	122	146	162	208	202	281	242	372	282	488	322	654	362	942
3	3	43	45	83	93	123	147	163	209	203	283	243	374	283	492	323	659	363	952
4	4	44	47	84	94	124	148	164	211	204	285	244	377	284	495	324	664	364	963
5	5	45	48	85	96	125	150	165	213	205	287	245	379	285	499	325	670	365	974
6	6	46	49	86	97	126	151	166	214	206	289	246	382	286	502	326	675	366	986
7	7	47	50	87	98	127	153	167	216	207	292	247	384	287	506	327	680	367	998
8	8	48	51	88	99	128	154	168	218	208	294	248	387	288	508	328	686	368	1010
9	9	49	52	89	101	129	156	169	220	209	296	249	390	289	513	329	692	369	1023
10	10	50	53	90	102	130	157	170	221	210	298	250	392	290	516	330	697	370	1036
11	11	51	55	91	103	131	159	171	223	211	300	251	395	291	520	331	703	371	1050
12	12	52	56	92	105	132	160	172	225	212	302	252	398	292	524	332	709	372	1064
13	13	53	57	93	106	133	162	173	227	213	304	253	400	293	527	333	715	373	1078
14	14	54	58	94	107	134	163	174	228	214	306	254	403	294	531	334	721	374	1093
15	15	55	59	95	108	135	165	175	230	215	308	255	406	295	535	335	727	375	1109
16	16	56	60	96	110	136	166	176	232	216	311	256	409	296	539	336	733	376	1125
17	17	57	61	97	111	137	168	177	234	217	313	257	411	297	543	337	739	377	1142
18	18	58	63	98	112	138	169	178	236	218	315	258	414	298	547	338	746	378	1160
19	19	59	64	99	114	139	171	179	237	219	317	259	417	299	551	339	752	379	1179
20	21	60	65	100	115	140	172	180	239	220	319	260	420	300	555	340	759	380	1198
21	22	61	66	101	116	141	174	181	241	221	322	261	423	301	559	341	766	381	1219
22	23	62	67	102	118	142	175	182	243	222	324	262	426	302	563	342	772	382	1241
23	24	63	69	103	119	143	177	183	245	223	326	263	429	303	567	343	779	383	1263
24	25	64	70	104	120	144	179	184	246	224	328	264	432	304	571	344	786	384	1288
25	26	65	71	105	122	145	180	185	248	225	231	265	434	305	575	345	793	385	1314
26	27	66	72	106	123	146	182	186	250	226	333	266	437	306	579	346	801	386	1341
27	28	67	73	107	125	147	183	187	252	227	335	267	440	307	584	347	808	387	1371
28	29	68	75	108	126	148	185	188	254	228	338	268	443	308	588	348	816	388	1403
29	30	69	76	109	127	149	186	189	256	229	340	269	447	309	592	349	824	389	1438
30	31	70	77	110	129	150	188	190	258	230	342	270	450	310	597	350	832	390	1476
31	32	71	78	111	130	151	190	191	260	231	345	271	453	311	601	351	840	391	1518
32	33	72	79	112	131	152	191	192	262	232	347	272	456	312	606	352	848	392	1565
33	34	73	81	113	133	153	193	193	263	233	349	273	459	313	610	353	857	393	1619
34	36	74	82	114	134	154	194	194	265	234	352	274	462	314	615	354	865	394	1681
35	37	75	83	115	136	155	196	195	267	235	354	275	465	315	620	355	874	395	1754
36	38	76	84	116	137	156	198	196	269	236	357	276	468	316	624	356	883	396	1844
37	39	77	86	117	138	157	199	197	271	237	359	277	472	317	629	357	892	397	1961
38	40	78	87	118	140	158	201	198	273	238	362	278	475	318	634	358	902	398	2127
39	41	79	88	119	141	159	203	199	275	239	364	279	478	319	639	359	911	399	2427
40	42	80	89	120	143	160	204	200	277	240	367	280	482	320	644	360	921	400	*

All holes must be clean and open.

* Indicates quantitative limit of state (approx 2628 particles) is exceeded.

"positive hole" method or by the microscope method. The positive hole method is essentially a count of the jets which delivered viable particles to the petri plates and the conversion of this count to a particle count by use of the "positive hole" conversion table (table 1). This table is based upon the principle that as the number of viable particles being impinged on a given plate increases, the probability of the next particle going into an "empty hole" decreases. For example, when 9/10 of the holes have each received 1 or more particles, the next particle has but 1 chance in 10 of going into an empty hole. Thus, at this point, on the average, 10 additional particles would be required to increase the number of positive holes by 1, and before all the holes become positive, some holes will receive a number of particles. The values in the table were calculated from the basic formula (Feller, 1950):

$$P_r = N\left[\frac{1}{N} + \frac{1}{N-1} + \frac{1}{N-2} + \cdots \frac{1}{N-r+1}\right]$$

Where P_r is the expected number of viable particles to produce r positive holes and N is the total number of holes per stage (400). The above formula assumes that the flow of particles stops at the instant a particle enters the rth hole. Since, in the actual case of sampling, the flow of particles stops at random, the expected number of particles present if r positive holes are observed, would be equal to or greater than P_r but less than P_{r+1} and the average would be $(P_r + P_{r+1}^{-1})/2$. This correction has been applied in the construction of the table.

In using the positive hole conversion table the number of positive holes must be precisely determined. A colony out of the hole pattern is not counted as a positive hole. By this method, counts up to 1200 or 1500 particles per stage are quite reliable. If higher counts are to be encountered, the microscope method is employed. With this method, the number of viable particles per stage is determined after a short incubation period by counting, with the aid of a microscope, the microcolonies in a number of deposit areas and calculating the total for the plate. A deposit area is that area which receives particles from one jet or hole. The microcolonies must be counted before they merge and, if done at the right time, as many as 20 or 30 per deposit area can be counted. By this method, total sampler counts as high as 40,000 or 50,000 can be made. For work that requires higher counts, an aerosol diluter may be employed with ratios up to 1:20. This diluter, the subject of a later paper, is a device attached to the intake of the sampler, which filters a known proportion of the air entering the sampler.

EXPERIMENTAL METHODS

Some of the experiments reported in this paper were performed with original and developmental models of the sampler. This, however, should in no way detract from the quality or reliability of the data because the fundamental principles upon which the sampler functions have been the same in all models. From the first to the present model, improvements have been made to increase the capacity, to decrease the size and weight, to simplify fabrication, and to make handling easier.

Wall loss. Impaction of particles anywhere within the sampler other than on the medium is defined as wall loss. The instrument was designed to minimize this. There are no dead air spaces and the velocity of the air moving through the device is greatest in the jets, and at all other points the velocity is not enough to carry the particles to any surface. Laboratory experiments were performed, however, to determine the possibility and extent of wall loss. Aerosols of *Bacillus subtilis* (morphotype *globigii*) and *Serratia marcescens* containing thousands of particles were drawn into the sampler. The device was immediately disassembled and swabbed at 40 locations on its inner walls and on the exteriors of the petri dishes where contamination was most likely. The experiments were repeated by a second worker for confirmation and elimination of possible bias. In no instance were any organisms recovered from the swabs.

In further experiments, an aerosol containing an extremely large number of particles (22×10^{10} cells) was drawn into the sampler. Forty-seven areas within the sampler were swabbed. Despite exposures of the sampler to this tremendous number of organisms, more than one-third of the swabs were negative and the others yielded only low counts (1 to 29). These results indicate that wall loss is extremely low. It therefore follows that with proper handling and operation the sampler does not become soiled or contaminated and, except for very special detection studies or work with pathogenic aerosols, it does

TABLE 2

Results from sampling Serratia marcescens aerosols with various sampler stage arrangements at a flow rate of 1 cu ft per min

Stage No.	Andersen-Sampler Stage Arrangement							
	1		2		3		4	
	Diameter of holes	Particle count	Diameter of holes	Particle count	Diameter of holes	Particle count	Diameter of holes	Particle count
	in		*in*		*in*		*in*	
1	0.052	1	0.052	2	0.052	9	0.052	0
2	0.039	4	0.024	134	0.024	116	0.024	106
3	0.031	27	0.0135	363	0.024	43	0.0135	290
4	0.024	73	0.0135	25	0.0135	363	0.010	35
5	0.0135	317	0.0135	25	0.0135	20	0.006	0
6	0.010	3	0.010	44	0.0135	11	MF*	0
7	0.006	0	0.006	0	0.010	3		
8	MF*	0	MF*	0	0.006	0		
					MF*	0		

* Millipore filter.

not require cleaning or sterilization between runs. This has been verified by negative control plates following collection of positive samples.

Glass, aluminum, and plastic petri dishes. The use of glass, aluminum, and disposable plastic petri dishes in the sampler was investigated. It was found that glass and aluminum petri dishes yielded about equivalent counts. The plastic dishes consistently gave lower counts, which averaged about 20 per cent less than counts secured with glass or aluminum dishes in 9 trials with each type of dish.

Further experiments showed that this lower count in the plastic dishes was due to the retention of aerosol particles on the exterior of the plastic dishes and on the walls of the sampler itself when plastic dishes were used in it. This phenomenon was probably caused by the electrostatic charge on the plastic dishes. The use of plastic dishes was therefore abandoned. The opaqueness of the aluminum dishes, which made observation and counting of colonies difficult, seemed to more than offset the advantage of unbreakableness and, therefore, use of the aluminum dishes was also abandoned.

Slippage. In the first model of the Andersen sampler, a Millipore filter was used as a final stage primarily to detect slippage, which may be defined as the passage of viable bacterial particles completely through the sampler. To determine the necessary combination of jet sizes that will insure complete collection of all the bacterial particles, fine aerosols of *S. marcescens* were generated and collected in samplers consisting of various stages as indicated in table 2. In all cases the final four stages consisted of three having jets of 0.0135, 0.010, and 0.006 in in diameter, respectively, and a Millipore filter. The results of these experiments are listed in table 2. It can be seen that only a very small percentage of particles got past the stage containing jets 0.0135 in in diameter, and none got past the stage containing 340 jets 0.010 in in diameter.

With a flow-rate of 1 cfm the calculated jet velocities for 340 hole stages are: 49.26 ft/sec for 0.0135 in holes and 90.75 ft/sec for 0.010 in holes. From these calculations and the results listed in table 2 it can be seen that a sampler with a jet velocity in the final stage of slightly more than 49 ft/sec would be adequate to prevent any slippage. Thus, the present model, figures 1 and 2, with stages containing 400 holes and having a jet velocity of 76.40 ft/sec in the final stage has a good margin of safety against slippage of *S. marcescens* or other similarly small microbial particles.

Humidification of air within the sampler. In addition to having no wall loss or slippage in a bacterial aerosol sampler, it is essential that desiccation of the viable particles it collects be prevented. Although the air entering the sampler described here may be quite dry, the relative humidity within the sampler rapidly increases. In one typical experiment, the air entering the

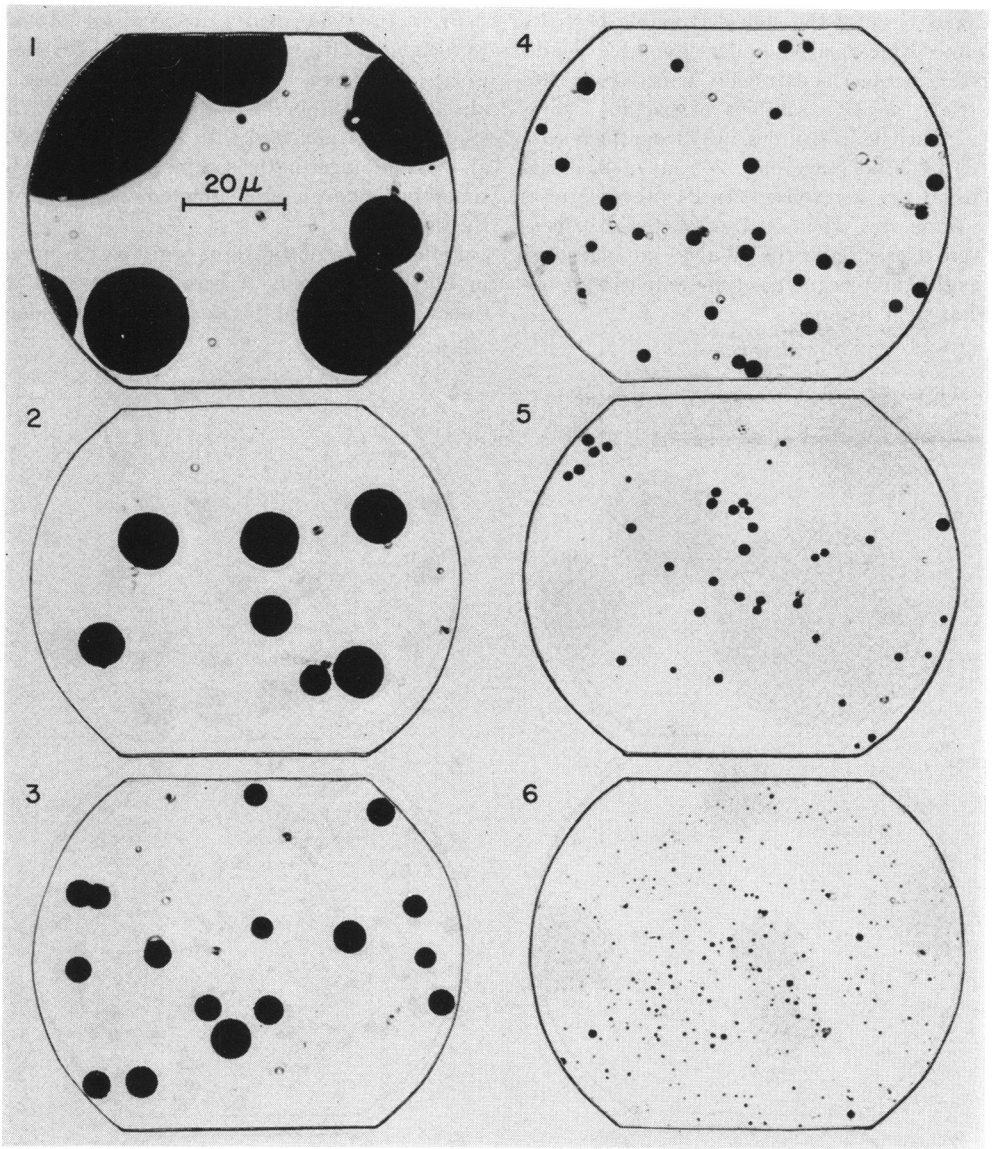

Figure 3. Photomicrographs of particles of aerosolized Carnauba wax as collected on the six stage of the Andersen sampler.

sampler had a relative humidity of 23 per cent, and after each stage, it had the following percentages: first, 39; second, 54; third, 67; fourth, 77; fifth, 85; and sixth, 88. This increase in the relative humidity makes conditions ideal for survival of the viable particles during the sampling period. Experiments have shown that drawing air through the sampler for 1 hr following collection of an *S. marcescens* sample does not reduce the count. Where the collected particles are smallest and most susceptible and jet velocities are greatest, the relative humidity and protection against desiccation is greatest. This increase in relative humidity plays an important role in the collection of biological or other hygroscopic particles. The lack of this feature in the single stage sieve sampler is probably responsible for both the slippage and the killing exhibited in that device.

Sensitivity. Because there is no slippage and no detectable wall loss with the usual sampling

loads, and because the collected viable particles are immediately placed under favorable conditions, this sampler is extremely sensitive. In two field trials, positive samples of bacillus spores were obtained 38 to 40 miles away from the release point. In the one case only 200 ml of bacterial suspension was aerosolized. In 24 previous tests in the same area when 50 L of bacterial suspension were released and the all-glass impinger was used as the sampler, no positive samples were obtained at such distances.

Prior to 1955, many unsuccessful attempts were made with various sampling devices to isolate the fungus *Coccidioides immitis* from the air at Camp Roberts, California. Similar field studies were conducted with the sampler and the all-glass impinger in the summer of 1955 at which time the organism was isolated, but only with the sampler.

Following an outbreak of ornithosis in humans in Portland, Oregon, it was demonstrated with tracer organisms and this sampler that a rendering

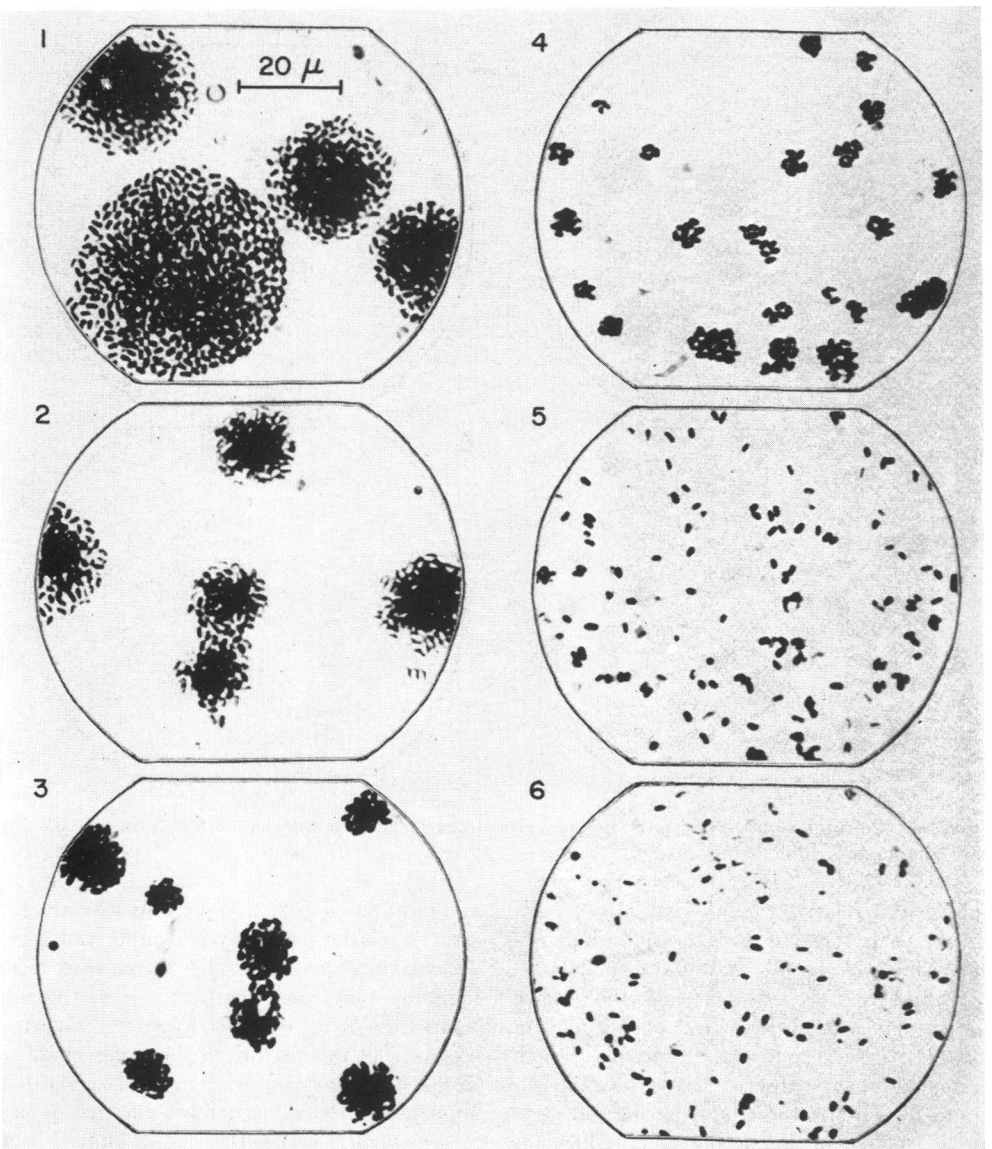

Figure 4. Photomicrographs of aerosol particles of *Bacillus subtilis* spores as collected on the six stages of the Andersen sampler.

TABLE 3

Size and stage distribution of particles of Carnauba wax and of Bacillus subtilis

Stage No.	Carnauba Wax Particles (Diameter Range in Microns)	*B. subtilis* Aerosol Particles (Cells per Particle* Range)
1	8.2 and larger	150 to 1000 or more
2	5.0 to 10.4	22 to 200
3	3.0 to 6.0	9 to 25
4	2.0 to 3.5	3 to 10
5	1.0 to 2.0	1 to 4
6	Up to 1.0	1

The ranges include 95 per cent or more of the particles collected on each stage.

* Cells per particle estimated for the larger particles where counts could not be made.

plant, processing diseased turkey carcasses, was generating aerosols inside and outside the building. Thus the source and mode of disease transmission for the ornithosis cases in and near the plant was indicated (Spendlove, 1957).

Particle size and stage distribution. To determine the size of particles collected on each stage of the sampler, studies were conducted with aerosols of Krylon, Carnauba wax, egg slurry, bacterial slurries, and other materials. Krylon aerosols were generated directly from the spray container. Carnauba wax aerosols were formed by aerosolizing the melted wax with an atomizer. Egg slurry and bacterial suspensions were aerosolized with a Chicago atomizer or Wells nebulizer. The nonviable materials all gave aerosols of spherical particles with smooth surfaces which were easily measured with a microscope equipped with a screw micrometer eyepiece. All of them reacted similarly in the sampler. Space will not permit the listing of all the data nor inclusion of photomicrographs of the particles of all of these materials; however, some of the data and photomicrographs secured with Carnauba wax and bacterial suspensions are presented.

The photomicrographs of wax particles containing ethyl violet and particles of stained spores of *B. subtilis* were made of the collected materials directly on the agar medium through the oil immersion objective. The petri plate was placed on the stage of the microscope, and the deposit area of impacted particles was located under low power objective and 10× ocular. A drop of oil was carefully placed on the area and the oil immersion objective (97×) turned into position. The photographs were made on 35 mm film and the six stage prints were mounted together in figures 3 and 4. The diameter of the wax particles was determined under oil immersion objective with a screw micrometer eyepiece. Because of the error in focusing the width of the lines, readings are not considered more accurate than ±0.2 microns. The wax particles remained spherical when impacted on the agar, but the spores of *B. subtilis* particles flattened out either from impaction or from the moisture of the medium until nearly all of the individual cells could be seen except those in the center of the larger clusters.

Table 3 summarizes, for each stage, the size data for the wax particles and gives estimates of cells for the aerosol of *B. subtilis*. Figure 5 characterizes the new 400-hole sampler as regards the particle size range and the stage distribution relationships.

Figure 6 illustrates how the sampler reflects the particle size spectrum of bacterial aerosols, and how the device may be used to study the effect of suspending medium on particle size.

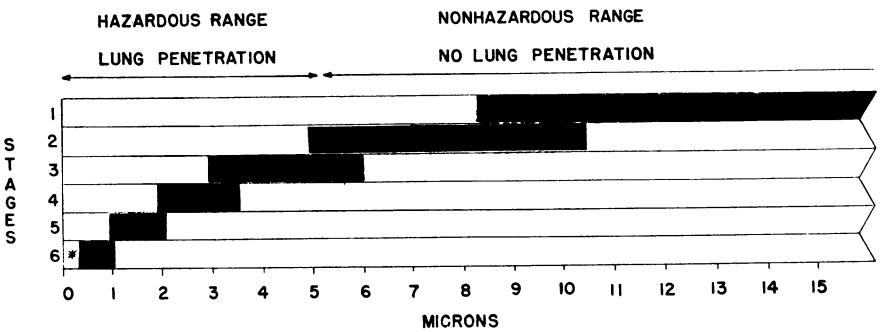

Figure 5. Relationship of stage distribution to particle size for smooth, spherical particles of unit density collected in the Andersen sampler. Each bar includes 95 per cent or more of the particles collected on that stage (*). The lower limit of stage six has not been determined.

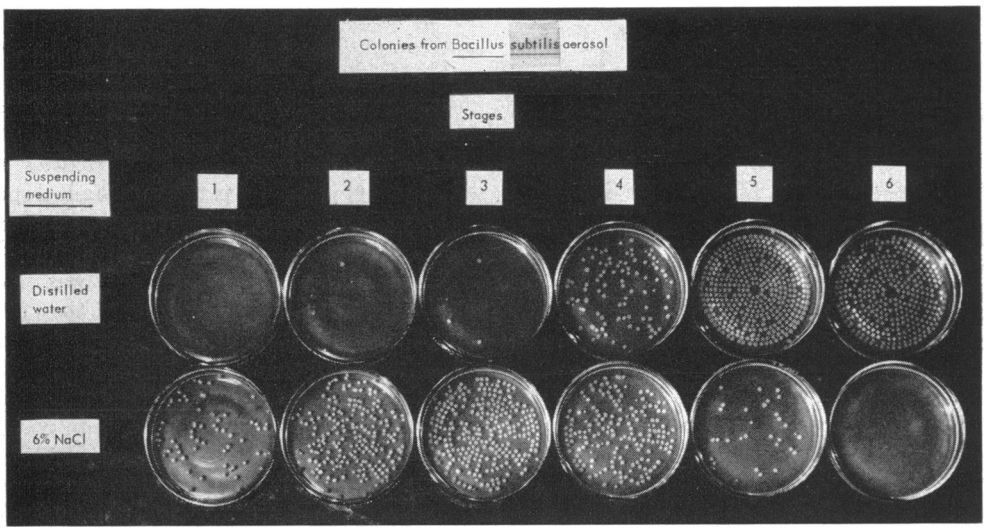

Figure 6. Effect of sodium chloride in the suspending medium on aerosol particle size spectrum as reflected by Andersen sampler stage distribution.

Effect of spacing distance upon stage distribution. The amount of medium poured in the petri dishes determines the distance between the jet openings and the impaction surface. Ranz and Wong (1952) recommended that this distance be within four jet diameters. This, however, would be quite impractical on the lower stages where jets are as small as 0.010 in in diameter and the stipulated spacing would then be 0.040 in or less. Spacing this small would be difficult to control very well with agar plates; moreover, it might increase the horizontal velocity of the air at the periphery of the plate so greatly that the proper operation of the device might be affected. Furthermore, it has been found that spacing less than 0.10 in (2.5 mm) is unnecessary.

Several experiments were conducted to determine the effect of varying the amount of medium on stage distribution. Plates containing 24, 27, and 30 ml were used. Spacing for these amounts of medium was approximately 3.0, 2.5, and 2.0 mm. The data from one typical experiment, consisting of nine samples, are presented in table 4. This table indicates that only a gradual shift in stage distribution resulted from thus varying the amount of medium with its consequent effect on spacing. This seems to indicate that spacing is not as critical as was first thought. On the basis of these data and Ranz and Wong's recommendation, and also upon practical considerations, 27 ml was selected as the optimum amount of medium to be used in the new molded petri dish[4] designed especially for use in this sampler.

TABLE 4

Effect of varying the amount of medium or spacing upon stage distribution of collected particles

Medium	Percentage of Particles Collected on Each Stage*					
	1	2	3	4	5	6
ml						
24	2.9	14.1	44.5	30.5	7.9	0.1
27	3.1	14.7	50.0	26.2	6.0	0.0
30	3.7	15.0	53.0	23.2	5.1	0.0

* Each value is the average of three trials.

Decay rate of bacterial aerosols. Since the sampler separates airborne particles into various size categories, it is uniquely useful for studies of bacterial aerosol decay. This feature of the device was effectively demonstrated in a series of experiments conducted with the original four stage (plus Millipore filter) model of the sampler. Aerosols of *B. subtilis* were generated into a 350 cu ft cubical chamber. Samples of these aerosols were taken with both the Andersen sampler and the standard all-glass impinger 5 min after genera-

[4] This special petri dish is fabricated by Corning Glass Works, with whom large orders may be placed directly. Any quantity is available from the author, 1074 Ash Ave., Provo, Utah.

tion and at 15 min intervals thereafter for 2 hr. The aerosols were kept uniformly dispersed in the chamber during the experiments by four fans operated at slow speed. Figure 7 is a photograph of the plates from one experiment, and table 5 lists the data from the same experiment. The sampling data are corrected to represent 1 cu ft samples for each device.

Sampler particle counts compared with impinger cell counts. The sampler results represent viable particles, whereas the all-glass impinger results represent viable cells (Druet *et al.*, 1953). In order to make a valid comparison of these two instruments, very fine aerosols of *B. subtilis* and *S. marcescens* produced with a Wells nebulizer were sampled with each instrument. If the viable particles of these aerosols were single cells, the two counts should be the same. The aerosols were drawn through a glass tube equilibrating chamber (4 in in diameter by 4 ft long) by the two

Figure 7. Plates from a decay rate study. An aerosol of *Bacillus subtilis* generated in a chamber was sampled at 5 min and each 15 min thereafter for 2 hr with the Andersen sampler. Each vertical row represents a sample collected in a four-stage sampler.

TABLE 5

Particle counts from Andersen sampler and cell counts from all-glass impinger in aerosol decay rate study

Stage	Age of Aerosol when Samples Were Taken (Min)								
	5	20	35	50	65	80	95	110	125
Sampler particle-counts									
1st	87	2	0	0	0	1	0	0	0
2nd	692	12	3	1	0	1	1	1	0
3rd	1029	207	68	23	5	5	3	2	0
4th	168	185	128	120	62	47	25	22	14
MF*	0	0	0	1	1	0	0	1	0
Total particles per cu ft	1976	406	199	145	68	64	29	26	14
All-glass impinger cell counts									
Total cells per cu ft	909	205	112	63	9	26	39	0	0

* Millipore filter.

TABLE 6

Comparison of the Andersen sampler particle counts with the all-glass impinger cell counts of Bacillus subtilis and Serratia marcescens

B. subtilis				*S. marcescens*			
Expt no.	Sample no.	Andersen sampler particle count*	All-glass impinger cell count	Expt no.	Sample no.	Andersen sampler particle count	All-glass impinger cell count
1	1	3,389	2,360	1	1	5,160	615
	2	3,380	1,552		2	5,796	1,265
					3	6,528	771
2	1	1,340	440		4	4,248	1,794
	2	1,320	363		5	8,328	2,859
	3	1,330	399		6	8,028	2,515
	4	1,380	250				
				2	1	21,924	2,489
3	1	996	992		2	21,132	3,365
	2	816	672		3	22,020	7,735
	3	1,032	640		4	21,852	10,732
					5	23,568	11,572
4	1	2,136	1,400		6	20,004	10,093
	2	2,220	1,530				
	3	2,148	1,122	3	1	2,760	3,038
					2	5,016	1,935
					3	5,652	4,108
					4	4,824	3,603
					5	4,632	2,470
					6	4,404	2,115
				4	1	8,292	11,900
					2	22,056	12,950
					3	34,332	11,138
Totals		21,478	11,720			260,556	109,062
Per cent		100	55			100	42

* Counts have been corrected to represent equivalent samples in the two devices.

sampling devices, alternately. The size of the samples was determined by the length of time the nebulizer was functioning. For each sample, after the nebulizer was shut off, clean air was drawn through the tube to sweep all the particles generated into the sampling device. In order to obtain optimum size samples for each device, the nebulizer was run 30 sec for the sampler and 6 min for the impinger.

Twelve samples of *B. subtilis* and twenty-one samples of *S. marcescens* were taken with each instrument, and the results are given in table 6. The impinger counts have been corrected to represent the total number of cells collected, and the sampler counts have been corrected to represent a sampling time equal to that of the impinger. The impinger cell count was 55 per cent of the corrected particle count for *B. subtilis* and 42 per cent for *S. marcescens*.

It is difficult to account for the differences exhibited by these two sampling devices, especially the bacillus spore count. It has been the experience of this laboratory that the action of the standard all-glass impinger reduces the count of both vegetative cells and spores.

DISCUSSION

The design of the sampler, the number and combination of stages, the number and size of the jets, and the sampling flow rate may be modified to meet the requirement of special problems.

The sampler and the respiratory tract function in a similar manner in retaining airborne particles. Particles 5 microns and larger are retained in the

upper respiratory tract (Brown *et al.*, 1950). This same fraction is collected on the first two stages of the sampler. Penetration in the respiratory tract increases with decreasing particle size (ibid), likewise in the sampler. The 1 micron particles retained in the respiratory tract are all found in the alveoli (ibid) and in the sampler they are found on the two lower stages.

If, according to Wells (1955), it is the aerodynamic dimension of the particle rather than the size that we should be concerned with in respiratory penetration studies, then it would be well to calibrate the sampler in terms of smooth, spherical particles of unit density, so that all particles collected in the sampler, regardless of their size, shape, density, etc., could be assigned an "effective size" or aerodynamic dimension according to the stage on which they were collected. This assigned value would be equal to that of the spherical particles collected on the same stage. Carnauba wax was selected for the calibration of the sampler because its density is very near 1 (0.995 to 0.999) and it is readily aerosolized into smooth, spherical, solid particles of measurable diameters.

The overlapping of particle size between stages, which is naturally inherent in all cascade impaction devices is minimized in this sampler by design. Ranz and Wong (1952) stated that as a particle passes through a jet its nearness to the axis of the jet is one of the factors that determines whether or not the particle will reach the impaction surface. In contrast to samplers which have one large rectangular jet in each stage, such as the cascade impactor (May, 1945) the Andersen sampler has 400 small, round jets. Travel of the particle is thus confined to near the axis of the jets. The average distance of the particles from the axis of the jets is very much less than in the cascade impactor. Ranz and Wong (1952) also stated that round jets have sharper cut-offs than rectangular jets. The Andersen sampler, therefore, on a theoretical basis, should have a sharper cut-off.[5] The experimental results, based on thousands of measurements, indicate that operation of the sampler is well within the limits of theoretical considerations based on application of Ranz and Wong (1952) and Davies and Aylward (1951).

In addition to the number and size of particles, it may be desirable to know the number of viable cells in the particles. This can be done with the Andersen sampler by collecting duplicate samples and using one set of plates for particle counts and the other set for cell counts. The cell count may be obtained by immediately washing the collected material from each plate into a flask, shaking the flask vigorously, and pouring the contents through a membrane filter. Dividing the cell count obtained from the membrane filter by the particle count of the corresponding plate of the other sampler gives the average number of viable cells per particle for each particle size category.

The Andersen sampler, as compared operationally with the all-glass impinger, saves both labor and expense: petri plates are filled and placed in the sampler, the sample drawn, and the plates incubated and counted. There is no plating process, and consequently, there are no pipettes and spreaders to wash, wrap, plug, and sterilize. There are no dilution tubes, impingers, and preimpingers to wash and prepare. With the Andersen sampler a little more time may be required for actual counting, but not only is considerable expense for labor and materials eliminated, but the results are obtained much sooner, and are much more meaningful.

Although the original intention was primarily to make a bacterial sampler, the device efficiently collects, in various size categories, all particulate airborne matter larger than a small fraction of 1 micron. This includes other microbial particles such as yeasts and molds, and nonviable particles such as dust, smoke particles, and pollens. Agar medium has been found to be an excellent collecting surface for these particles. Microscopic examination of collected material can be made directly on the agar plate under low, high, or oil immersion objectives. Various reagents for identification of specific particles might be put on or in the agar medium. Collected materials such as viruses or toxins can be washed off for animal injection or chemical analysis. Collection of aerosolized bacterial phage directly on seeded plates has been effectively demonstrated. The sampler should provide an efficient means for collecting, concentrating, and sizing radioactive particles for Geiger counter readings.

Because of its sensitivity in the detection of aerosols, the Andersen sampler should be useful for the detection and identification of pathogenic organisms which would be of much value in controlling sources of airborne disease. The elimina-

[5] A separate paper dealing with particle size cut-off in the Andersen sampler is in preparation.

tion of the plating process and the shorter incubation period would considerably lessen the time for identification and therefore would permit earlier treatment of exposed populations.

ACKNOWLEDGMENTS

The author wishes to acknowledge the assistance of H. B. Allen, E. J. Blommer, and M. L. Southworth in the collection of some of the data, and J. M. Turnquist and P. A. Peck in mathematical and statistical problems. The author also wishes to thank R. A. McCullough for editorial assistance and Drs. T. F. Dozois and J. C. Spendlove, Lt. Col. C. W. Meyers, and numerous other persons for encouragement, interest, and helpful criticism.

SUMMARY

A new type of aerosol sampler that collects airborne particles in several categories of decreasing particle size is described, and procedures for its operation are given.

Experimental studies with the instrument showed that, (a) wall loss is negligible, (b) there is no slippage of bacterial particles, (c) the instrument is extremely sensitive, and (d) particle size discrimination makes it possible to calculate the particle size spectrum of bacterial aerosols.

It is also suggested that the sampler provides a suitable means of assessing the health hazard in particulate air pollution.

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